

Cementum-Forming Cells Are Phenotypically Distinct from Bone-Forming Cells

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ABSTRACT

Normal human cementum-derived cells (HCDCs), expanded *in vitro*, formed mineralized matrix when attached to a ceramic carrier and transplanted subcutaneously into immunodeficient mice. The mineralized matrix elaborated by transplanted HCDC exhibited several features identical to cementum *in situ* and was significantly different from bone deposited by similarly transplanted human bone marrow stromal cells (BMSCs). No bone marrow formation and very few or no tartrate-resistant acid phosphatase (TRAP)-positive cells (osteoclasts and osteoclastic precursors) were found in HCDC transplants. In contrast, in BMSC transplants both hematopoiesis and TRAP-positive cells were routinely observed. Furthermore, compared with BMSC-derived matrix, HCDC-derived matrix was less cellular, numerous empty lacunae were present, and fewer cells were found on the cementum matrix/ceramic carrier interface. The organization of collagen fibers in HCDC-derived matrix, as visualized by using the Picrosirius red staining method, was similar to cementum, with typical unorganized bundles of collagen fibers. In contrast, bone matrix elaborated by transplanted BMSC had lamellar structure, identical to mature bone *in situ*. Finally, cementocytes embedded in the cementum-like matrix were immunopositive for fibromodulin and lumican, whereas osteocytes within the bonelike matrix were negative. This pattern is consistent with the cementum and bone *in situ*, respectively. These results indicate that human cementum cells are phenotypically distinct from bone cells and provide further validation of the combined *in vitro/in vivo* model of human cementogenesis recently developed in our laboratory. (J Bone Miner Res 2000;15:52–59)

Key words: cementum, bone, cementoblasts, osteoblasts, cementogenesis

INTRODUCTION

TOOTH CEMENTUM is generally thought to be closely related to bone.^(1,2) Both cementum and bone are mineralized tissues with a comparable mineral-to-organic matrix ratio, although some minor differences in this parameter have been reported in the literature.^(1,2) The organic matrix of both tissues is composed mostly of type I collagen (approximately 90% of the organic component) with a similar set of noncollagenous matrix proteins (NCPs).^(1–7)

The most abundant NCPs include osteopontin, bone sialoprotein, osteocalcin, fibronectin, and several species of proteoglycans, such as decorin (DCN), biglycan (BGN), lumican (LM) and fibromodulin (FM).^(1–12) Although the known compositions of cementum and mature bone are similar, their morphological appearances differ. Mature bones (both cortical and trabecular) in humans have a lamellar organization, whereas cementum does not but rather resembles a primitive, fetal-type woven bone.^(1,2,12–15) Bone, with few exceptions, is accompanied by bone marrow. No bone marrow is

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present within or around cementum. In addition, cementum is avascular and does not undergo any remodeling in normal conditions, whereas bone contains extensive vasculature and is constantly remodeled throughout life.^(1,2) Furthermore, qualitative and/or quantitative differences in the composition of the organic component between bone and cementum may exist because in vitro cellular responses to the cementum matrix appear to be different from the responses invoked by bone matrix.⁽¹⁶⁻¹⁹⁾ However, it is still unclear whether these distinctive features of cementum are caused by its specific location and the influence of neighboring tissues, most notably periodontal ligament (PDL) and dentine,^(5,19,20-22) or whether they are intrinsic to the cementoblastic phenotype.

At present, only a few poorly characterized molecules (cementum attachment protein, CAP; and cementum-derived growth factor, CDGF) have been postulated to be cementum specific.^(16,17) This needs further confirmation because no protein or messenger RNA (mRNA) sequences of these proteins are available at present.⁽¹⁷⁾ Our recent studies have indicated that two small keratan sulfate proteoglycans, FM and LM, are more abundant in cementum than in bone,⁽¹⁰⁾ with specific localization restricted to unmineralized cementoid and around cementocytes. Thus, it is conceivable that these small keratan sulfate proteoglycans (LM and FM) are involved in cementum physiology.

Because of the critical role of cementum in the structure of the periodontium and the high prevalence of periodontal disease, there is great interest in the physiology of cementum. Despite its clinical relevance, the goal of developing efficient, safe, and reproducible strategies to regenerate cementum has not been accomplished yet to a degree similar to that in bone.⁽²³⁾ The major obstacle has been the paucity of available models to study human cementogenesis in general and lack of appropriate in vitro models in particular.^(8,20,24) It is still unclear whether cementum cells (cementoblasts and cementocytes) are a distinct lineage or whether they belong to the same lineage as bone cells (osteoblasts and osteocytes), and the observed differences between bone and cementum are attributable to the specific anatomical localization ("positional effect"⁽²⁰⁾). Furthermore, the location and origin of cementoblastic progenitors/precursors that differentiate into functional cementoblasts in the fully developed tooth remain speculative.⁽¹⁹⁾

Recently, we have established a method to isolate and expand in vitro normal human cementum-derived cells (HCDCs). Some of these HCDC strains formed mineralized, bone/cementum-like tissue when transplanted into immunodeficient mice.⁽²⁴⁾ In the present report, after more extensive testing, we provide several lines of evidence that the organization and composition of the mineralized tissue elaborated by transplanted HCDCs are identical to cementum in situ. In addition, this tissue differs significantly from that derived from transplanted osteoprecursor cells (bone marrow stromal cells, BMSCs), and from mature bone in situ. Thus, the cementum-forming cell (cementoblast) may represent a unique cell that is substantially different from cells of osteoblastic lineage.

MATERIALS AND METHODS

Cell culture

Normal HCDCs: Healthy human premolar teeth (from patients aged 12–14 years) extracted for orthodontic reasons were used. The "human subjects" protocol was approved by the Committee on Investigations Involving Human Subjects, School of Dentistry, University of North Carolina. Teeth were either kept in serum-free Dulbecco's modified Eagle medium (DMEM)/F12 medium (Gibco-BRL, Life Technologies, Grand Island, NY, U.S.A.) containing 100 U/ml of penicillin and 100 µg/ml of streptomycin (Gibco-BRL) overnight at 4°C or processed immediately after extraction.

The cultures of HCDCs and control PDL cells were established as described.⁽²⁴⁾ Briefly, PDL was manually dissected from the tooth root with a surgical scalpel and used to establish cultures of PDL cells. After extensive washing with plain medium, teeth were subjected to collagenase P (100 mU/ml; Boehringer-Mannheim, Mannheim, Germany) treatment for 1.5–2 h at 37°C. The medium with released cells was discarded, and cementum, together with a thin layer of underlying dentin, was dissected and collected. These fragments were thoroughly washed, then minced with scissors until small chips (<0.5 mm in diameter) were obtained. The chips were next digested again with collagenase P for 1.5 h at 37°C to remove all cells unprotected by mineral. After this step, the fragments were washed thoroughly with medium and placed in 150-mm tissue culture plastic Petri dishes (Costar, Cambridge, MA, U.S.A.) containing growth medium (DMEM/F12 supplemented with penicillin/streptomycin and 10% fetal bovine serum [FBS; Gibco-BRL]). The cultures were incubated in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Medium was changed every other day.

Under the conditions used (i.e., plating chips derived from one tooth into one 150-mm plate), HCDCs form colonies that are separated by a large distance. When the cell number within a colony reached 400–500 (usually after approximately 4 weeks in culture), cells from individual colonies were scraped with a Pasteur pipette, transferred to the wells of 12-well tissue culture plates (Costar), and cultured in the growth medium until semiconfluent (single colony-derived strains [SCDSs]). Cells that remained on the original plate were further incubated until semiconfluent (usually up to 6–7 weeks) and passaged, thus providing multicolony-derived strains (MCDSs).

BMSCs: The bone marrow specimens used to initiate the cultures were obtained in the course of corrective surgery from patients with scoliosis, under institutionally approved procedures for the use of human surgical waste. Cultures were established from bone marrow content of normal spine bone fragments as previously described,⁽²⁵⁻²⁷⁾ and MCDSs of human BMSCs that were age- and sex-matched to the HCDC strains were used in these studies. BMSC strains were extensively characterized for their bone-forming capabilities in an in vivo assay.^(25,26)

In vivo differentiation assay

Cementum-derived cells (both SCDSs and MCDSs) that have been previously assessed⁽²⁴⁾ for their potential to form mineralized matrix upon transplantation into immunodeficient mice were used. Human PDL cells and human BMSCs were used for comparison. It has been repeatedly demonstrated that in this system, the mineralized matrix formed around the ceramic particles is of donor origin and that the hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic carrier is permissive for several phenotypes (bone, cementum, fibrous tissue, marrow stroma, fat^(24–27)). If transplanted cells form significant amounts of bone, osteoclasts and bone marrow (both of recipient origin) settle within the grafts.^(25,26)

Throughout these studies, cells from the third passage were used. For a single transplant, 1.5×10^6 cells in 1 ml of growth medium were mixed with 40 mg of HA/TCP ceramic powder (Zimmer, Warsaw, IN, U.S.A.). The mixture was incubated at 37°C for 90 minutes with slow rotation (25 rpm) and centrifuged briefly, and supernatant was discarded. The pelleted HA/TCP powder with adherent cells was mixed consecutively with 15 μ l each of mouse fibrinogen (3.3 mg/ml solution in phosphate-buffered serum [PBS]) and mouse thrombin (25 U/ml in 2% CaCl_2 ; both from Sigma, St. Louis, MO, U.S.A.). The resulting fibrin clot with ceramic powder and cells inside was transplanted subcutaneously into 8–12-week-old female beige mice (NIH-bg-nu-xidBR; Harlan Sprague Dawley, Indianapolis, IN, U.S.A.) as described.^(24–27) Briefly, mice were anaesthetized by intraperitoneal injection with a combination of ketamine (Fort Dodge Animal Health, Fort Dodge, IA, U.S.A.) at 140 mg/kg body weight and Xylazine (The Butler Company, Columbus, OH, U.S.A.) at 7 mg/kg body weight. A single 1-cm-long skin incision was made on the dorsal surface of each mouse, and four subcutaneous pockets per mouse were created by blunt dissection. A single transplant was placed into each pocket, and incisions were closed with surgical staples.

The transplants were harvested after 9 weeks. Transplants were cut in half, fixed for 2 days in Bouin's solution (Sigma), and after 2 days decalcification in 10% formic acid, processed for routine histological examination (hematoxylin-eosin [HE] staining), immunohistochemistry, and Picrosirius red staining.

Morphological and immunohistochemical studies

Cellularity of the matrix and cellularity of the ceramic-matrix interface: The cellularity of the mineralized matrixes deposited by transplanted cells and the cellularity of the matrix-ceramic interface were evaluated on 15 randomly chosen, HE-stained sections through transplants. In total, matrices elaborated by two MCDSs of BMSCs, two MCDSs, and four (randomly chosen from seven) SCDSs of HCDCs were subjected to image analysis. Images were captured using an Olympus BH-2 microscope using the 10 \times objective (total magnification 256 \times) equipped with a video camera connected to an IBM-compatible PC computer using the Java software (Jandel Scientific, Corte Madera, CA, U.S.A.). Captured images were analyzed using the NIH

Image software (version 1.59) on a Power Macintosh 9500/132 computer. For each group of images, the area occupied by bone (or cementum)-like tissue was measured (pixel number; this value was further normalized to obtain the area occupied by mineralized matrix in mm^2), and nucleated cells embedded in the matrix area were counted. Cellularity of the matrix was calculated by dividing the total cell number within all the examined images by the total mineralized matrix area measured.

The cellularity of the mineralized matrix-ceramic interface was evaluated on the same images as the total cellularity of the matrix. The length of the matrix-ceramic interface was measured (pixel number, further normalized to the actual value in mm) using the NIH image software, nucleated cells on the interface were counted, and the cellularity of the ceramic-matrix interface was defined as cell number/1 mm of length of the interface. Only cells that were in physical contact with the ceramic carrier were counted.

The percentage of empty lacunae was evaluated in the following manner. Using a microscope at 400 \times magnification, 100 lacunae within the matrix were counted on random visual fields, including lacunae containing nucleated cells and lacunae devoid of cells; the ratio of empty lacunae to the total number of lacunae was calculated.

TRAP histochemistry: Eight transplants were frozen and then fixed with 4% paraformaldehyde, decalcified in 0.5 M ethylenediaminetetraacetic acid (EDTA) at 4°C and embedded in low-melting paraffin. Five-micrometer-thick sections were obtained and used for the histochemical localization of TRAP using a commercial detection kit (Sigma). Staining was performed according to the manufacturer's protocol.

Picrosirius red staining: Staining was performed as previously described.^(28,29) Briefly, specimens fixed with Bouin's solution were embedded in paraffin. Sections of relevant transplants 7 μ m thick and original human bone and teeth (age and sex matching the transplanted cells) were rehydrated and treated with 0.1% Sirius red (Sigma-Aldrich, Milwaukee, WI, U.S.A.) in saturated aqueous picric acid (pH 2.0) for 30 minutes. After a brief wash with 0.01 M HCl, sections were dehydrated and mounted in synthetic resin. Stained sections were evaluated in polarized light.

Immunohistochemistry: Deparaffinized sections of representative transplants, bone, and cementum were subjected to immunohistochemistry using polyclonal antibodies raised against the respective core proteins of LM (a generous gift of Dr. V. Hassell, Shriners Hospital, Tampa, FL, U.S.A.), FM (a generous gift of Dr. A. Plaas, Shriners Hospital, Tampa, FL, U.S.A.), DCN and BGN (a generous gift of Dr. Larry W. Fisher, National Institute of Dental and Craniofacial Research, Bethesda, MD, U.S.A.). Before immunostaining, rehydrated sections were treated with chondroitinase ABC (Seikagaku America, Ijamsville, MD, U.S.A.) to better expose the epitopes. After 2.5 h incubation with a 1:100 dilution of primary antibody and thorough washing with Tris-buffered saline (TBS), the sections were incubated for 30 minutes with alkaline phosphatase-conjugated secondary antibody (Pierce, Rockford, IL, U.S.A.) at the 1:200 dilution. After rinsing with TBS, color reaction was developed for 30 minutes with Vector Red substrate (Vector Laborato-

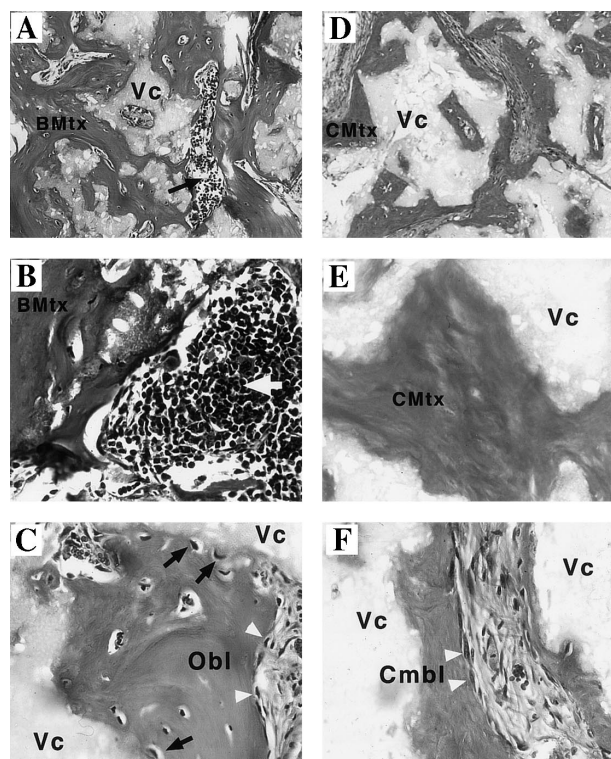


FIG. 1. Morphology of transplants containing (A–C) MCDSs of human BMSCs and (D–F) MCDSs of HCDCs. Original magnification (A, D) 40 \times ; (B, C, E, F) 300 \times . Note that the BMSC-derived matrix (A–C) appears to be more uniform and compact than the one deposited by HCDC (D–F). Note that bone marrow (black arrow in A, white arrow in B) is present in BMSC but not HCDC transplants. In C and F, black arrows indicate osteocytes on the ceramic–bone interface; white arrowheads indicate osteoblasts (C) and cementoblasts (F). Cementoblasts are larger and more elongated than osteoblasts (C, F). CMtx, cementum-like matrix; BMtx, bonelike matrix; Vc, area occupied by HA/TCP ceramic vehicle; Obl, osteoblasts; Cmbi, cementoblasts.

ries, Burlingame, CA, U.S.A.) and mounted using Vectashield (Vector Laboratories).

Statistics

All statistical analysis was performed using Student's *t*-test when appropriate.

RESULTS

All transplanted MCDSs of BMSCs and HCDCs formed mineralized tissue in the immunodeficient mice. Of the 10 SCDSs of HCDCs examined in this study, seven showed deposition of mineralized tissue after prolonged maintenance in immunodeficient mice (9 weeks); the remaining strains formed fibrous tissue only. By standard histology (HE staining), the mineralized tissue elaborated by trans-

TABLE 1. CELLULARITY, CELL NUMBER ON CERAMIC–TISSUE INTERFACE, AND PERCENTAGE OF EMPTY LACUNAE IN TISSUES ELABORATED BY TRANSPLANTED OSTEOGENIC BMSC AND CEMENTOGENIC HCDC

Cell type	Cells/1-mm interface	Cellularity of the matrix (cells/mm ² of matrix area)	% of empty lacunae
BMSC (2 MCDS)	7.2 \pm 1.1	187 \pm 18	9.1 \pm 2.2
HCDC (2 MCDS and 4 SCDS)	2.9 \pm 0.6*	135 \pm 26*	23.4 \pm 6.7*

Data represent means \pm SE.

*Significantly different from the value obtained for bone-like tissue; *p* < 0.05 (Student's *t*-test).

planted HCDCs was highly reminiscent of cementum (further referred to as “cementum-like tissue”). Overall, the amount of cementum-like tissue deposited by HCDC strains was less than the amounts of bone formed by BMSCs (approximately 60%), as evaluated in respective transplants 9 weeks after transplantation (Fig. 1). However, this parameter was variable, and approximately 20% of the SCDSs of HCDCs showed extensive cementum-like deposition, comparable to the amount of bone deposited by BMSC. It is unlikely that the observed differences among HCDC strains were due to the selective lack or impairment of resorption of the specific transplants because we did not detect significant numbers of TRAP-positive cells in any of the cementum-forming HCDC transplants, regardless of the amount of tissue deposited (see below).

Histologically, several differences were observed between tissues formed by HCDC and those formed by BMSC. First, no bone marrow developed in any of HCDC transplants, whereas hemopoiesis was observed routinely in BMSC transplants (Figs. 1a and 1b). Second, cementum cell-derived mineralized tissue consistently was less cellular than that formed by BMSC (Table 1, Figs. 1d–1f). In fact, some of the HCDC strains deposited mineralized tissue that resembled acellular cementum rather than cellular cementum (Fig. 1f). Third, in most transplants of HCDC, the cells lining the newly deposited mineralized matrix were consistently larger and more elongated than the corresponding cells in BMSC transplants (Figs. 1c and 1f). In addition, fewer cells were localized at the ceramic–mineralized tissue interface (again, consistent with the appearance of the cementum/dentin interface^(1,2)) than at the ceramic–bone interface in the tissue formed by BMSC (Figs. 1e and 1f; Table 1). Finally, few blood vessels were found within the mineralized cementum-like matrix, whereas in the bonelike matrix they were easily identified (Figs. 1e and 1f).

One of the characteristic features of the dentin–cementum interface is that it is virtually devoid of cells.^(1,2) This feature was maintained in the HCDC transplants as well. As shown in Table 1, in HCDC transplants the average cell number on the ceramic–matrix interface was approximately 40% of the number obtained for BMSC transplants. In addition, the HCDC-derived mineralized matrix was less cellular than the matrix elaborated by BMSC. On average, cementum-like

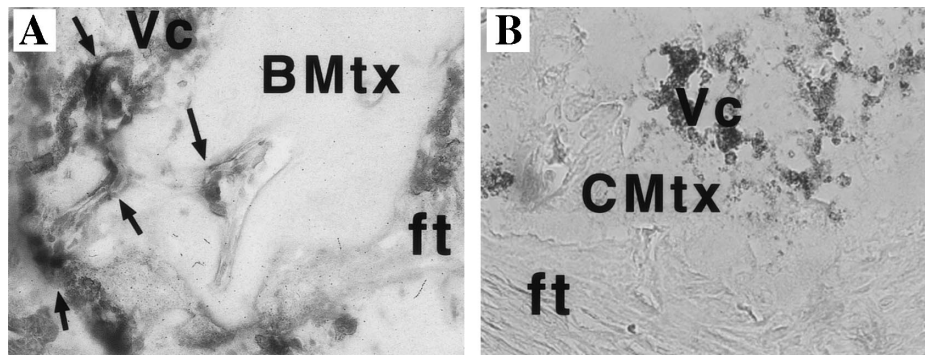


FIG. 2. Histochemical localization of TRAP (arrows) in representative (A) BMSC and (B) HCDC transplants (both MCDs). Original magnification 100 \times . Vc, vehicle; Bmtx, bone matrix; Cmtx, cementum matrix; ft, fibrous tissue.

matrix contained 72% of the number of cells obtained for bonelike matrix. Consistent with the histological picture of cementum in situ, numerous empty lacunae were observed within cementum-like matrix (Figs. 1c–1f; Table 1), but not within the bonelike matrix (Figs. 1a–1c; Table 1).

TRAP-positive cells (mononucleated osteoclastic precursors and mature, multinucleated osteoclasts) were readily visualized within BMSC transplants (Fig. 2a). Substantially weaker staining was visualized within fibrous tissue-forming transplants containing control PDL cells and HCDC strains that did not form any mineralized matrix (data not shown). In these transplants, the positive staining was localized mostly within foreign body cells in the proximity of the ceramic vehicle. All of the HCDC-containing transplants that formed mineralized tissue either showed only occasional TRAP-positive cells or were virtually devoid of any TRAP-positive cells, including the foreign body cell type (Fig. 2b).

At the light microscopic level and by standard HE staining, the organization of the matrix deposited by HCDC (Fig. 3B) appeared to be different from the matrix deposited by BMSC (Fig. 3A). To study the matrix organization in more detail, we used a Picrosirius red staining method that specifically enhances the natural birefringency of collagen fibers, particularly when visualized in the polarized light. The matrix formed by all strains of HCDC tested⁽¹²⁾ exhibited nonlamellar organization of collagen fibers (Fig. 3D) similar to that of cementum in situ (Fig. 3F). BMSC-derived mineralized matrix, on the other hand, resembled that of mature bone in situ (Fig. 3E), with a distinct lamellar and compact appearance (Fig. 3C).

We have recently shown that LM and FM are a major group of keratan sulfate proteoglycans in cementum.⁽¹⁰⁾ To further investigate the phenotypic features of the matrix elaborated by HCDC, we used the immunohistochemical approach to localize these two proteoglycans. As shown in Fig. 4, the area around cementocyte-like cells embedded in the matrix stained positively for both LM and FM (Figs. 4A and 4B). In contrast, osteocyte-like cells within the matrix elaborated by BMSCs were uniformly negative for FM and LM (Figs. 4C and 4F). Positive staining was observed within the connective tissue surrounding the mineralized matrices elaborated by both cell types (Figs. 4A, 4C, 4D, and 4F), indicating that less differentiated cells within fibrous tissue deposit both FM and LM. This pattern of staining for LM and FM was identical to that observed for these tissues (bone

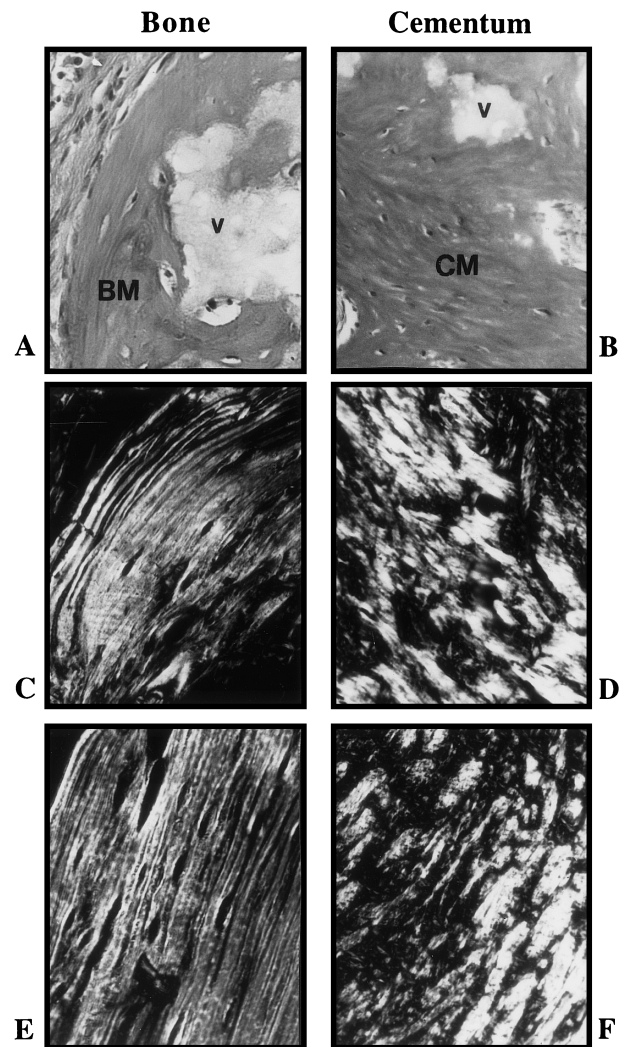


FIG. 3. (A) Bonelike tissue formed by transplanted BMSCs; (B) cementumlike tissue formed by transplanted HCDCs (SCDS C-11–26 12); (C) lamellar organization of collagen in bone deposited by BMSCs; (D) nonlamellar organization of the matrix deposited by HCDCs; (E) bone in situ; (F) cementum in situ. v, vehicle; BM, bone matrix; CM, cementum matrix. (A, B) HE staining, (C–F) Picrosirius red staining; images obtained under polarized light. Original magnification of all images 200 \times .

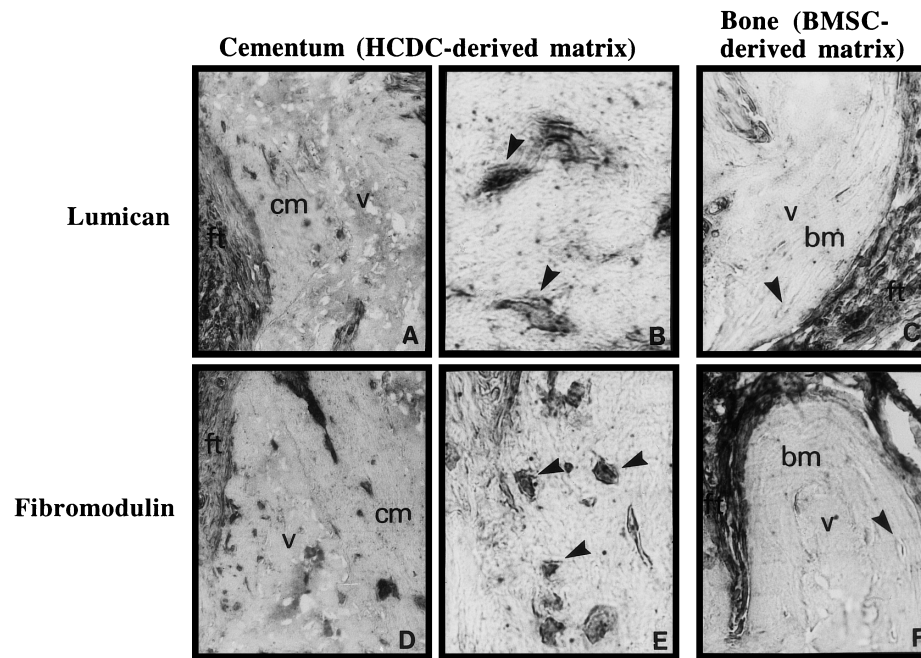


FIG. 4. Immunolocalization of FM and LM within transplants containing (A, B, D, E) cementum-derived cells and (C, F) bone marrow stromal cells. Original magnification (A, C, D, F) 100 \times ; (B, E) 400 \times . cm, cementum-like matrix; bm, bonelike matrix; ft, fibrous tissue; v, area occupied by HA/TCP ceramic vehicle. Arrowheads indicate cementocytes (B, E) or osteocytes (C, F).

and cementum) in situ.⁽¹⁰⁾ DCN and BGN were immunolocalized to both HCDC- and BMSC-derived matrices, with prominent pericellular localization, but there were no obvious differences in the staining pattern between the two tissues (data not shown).

DISCUSSION

Our laboratory has recently developed a combined in vitro/in vivo model to study normal HCDCs.⁽²⁴⁾ Some of the HCDC strains expanded in culture formed mineralized matrix when attached to a ceramic carrier and transplanted subcutaneously into immunodeficient mice. After more extensive studies and by use of new assays, in this report we provide further morphological, histochemical, and immunohistochemical data demonstrating that the mineralized matrix elaborated by transplanted HCDCs has several major features identical to the cementum in situ. In addition, the organization and composition of this matrix are different from those of the matrix deposited by transplanted osteoblastic precursors (BMSC). Specifically, HCDCs deposited collagenous matrix with an unorganized, nonlamellar appearance similar to that of cementum in situ. Such matrix exhibited significantly poorer cellularity, and fewer cells were present at the ceramic carrier–matrix interface than in the matrix elaborated by BMSCs. As in the cementum in situ, both FM and LM were present within and around cementocytes. Thus, our results further confirm the possibility that FM and LM play specific roles in cementum physiology, possibly by regulating the process of organization and subsequent mineralization of the newly deposited cementum matrix.⁽¹⁰⁾

The most striking feature of the transplants containing cementum-forming HCDC strains was that they were virtually devoid of any TRAP-positive cells, in contrast to the

BMSC transplants. It is still unclear whether these TRAP-positive cells visualized within the relevant transplants correspond only to osteoclasts or giant foreign body cells or to both because both cell types are multinucleated and can express TRAP, and distinguishing between these two populations outside of bone is not possible.⁽³⁰⁾ This reservation notwithstanding, our result is obviously consistent with the expected phenotype of cementum. It also suggests that cementum-forming HCDCs do not provide a proper environment for the recruitment and/or formation of TRAP-positive cells within the transplants. This feature, i.e., lack of osteoclast formation within bone formed by transplanted osteogenic cells, has been also reported for some bone marrow stromal cell lines; however, it is an exception rather than a rule in this setting.⁽³¹⁾ Thus, given the consistency of the observation that HCDC transplants did not support osteoclast formation, our results suggest that cementoblastic cells may be responsible for the lack of cementum remodeling in situ, possibly by preventing osteoclast recruitment and/or maturation.

Based on histological appearance, the cementum matrix resembles woven bone, showing typical, irregular bundles of collagen fibers. However, woven bone, in contrast to cementum, is always highly vascularized, osteocytes are large and abundant, and after development of bone marrow the preexisting woven bone is resorbed and replaced with mature, lamellar bone.^(1,2,21) Thus it is highly unlikely that the tissue formed by transplanted HCDCs was, in fact, woven bone. Lack of bone marrow, very poor vascularity, absence of osteoclastic cells, relatively low cellularity, and high numbers of empty lacunae are consistent with the histology of cementum but not of woven bone.

Our results clearly indicate that HCDCs isolated from cementum and expanded in vitro recapitulate most (if not all) of the basic features of human cementum formation

when transplanted into immunodeficient mice. Such HCDCs deposit cementum-like tissue in an ectopic site and in conditions devoid of any influence of relevant cell types that are normally present in periodontium. Furthermore, results presented in this report strongly support the hypothesis that cementum cells are different from osteoblastic cells. Under the conditions used (i.e., transplanted into the ectopic site of a xenogeneic recipient in a ceramic carrier), cells are deprived of any modifying influences derived from other cellular populations and/or matrix normally present in bone organ or in periodontium in situ. Yet in both cases, such unrestricted cellular differentiation resulted in the formation of distinctly different tissues that are highly reminiscent of the tissues of origin. This suggests that cementum-derived cells, once committed to this phenotype, are specifically programmed to deposit only cementum-like mineralized matrix, with a typical organization of this matrix and a specific set of molecules expressed (e.g., FM and LM). In contrast, osteoblastic precursors derived from bone marrow are committed to synthesize mature, lamellar bone when a hydroxyapatite-containing carrier is used. It is tempting to speculate that both cell types recapitulate the in situ deposition of the respective tissues, where mature lamellar bone is formed after resorption of the pre-existing woven bone, and cementum is deposited on the mineralized dentin. In fact, mineralized dentin is a prerequisite for cementum formation because factors that delay dentin mineralization also inhibit subsequent cementum formation.^(22, 32)

Our findings may have some important implications for development of strategies aimed at cementum regeneration through application of specific growth factors or via cell transplantation-based therapies. First, it appears that factors favoring extensive cementum deposition are intrinsically associated with the cells committed to such lineage. Factors that specifically stimulate cementum growth would be of obvious clinical relevance, and their application in cases with only a partial cementum loss seems logical. However, in the case of massive cementum loss, only cell transplantation therapies would be likely to succeed. Admittedly, cementum may not be a realistic source of cells to be expanded in vitro and retransplanted because it would require extraction of unaffected teeth. Thus, bone marrow stromal cells that contain a pool of less differentiated (or even noncommitted) mesenchymal stem cells are the most likely candidates to be effective in these settings.⁽³³⁻³⁵⁾ However, as our results show, their unrestricted differentiation in an immunodeficient mouse under the conditions used favors lamellar bone formation that is substantially different from that of cementum formed under identical conditions by cementum-derived cells. On the other hand, it is well established that BMSCs can be manipulated to a high degree both in vitro and in vivo so they can differentiate into fat, cartilage, or even muscle cells.⁽³³⁻³⁶⁾ In principle, then, it should be possible to establish conditions (either during the in vitro culture and/or by use of a special delivery vehicle) in which mesenchymal cells would differentiate into cells committed to formation of cementum. The combined in vitro/in vivo model system developed in the course of these studies should be an excellent tool to establish such conditions.

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